Detection of Ligand-Induced Conformational Changes in Oligonucleotides by Second-Harmonic Generation

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RNA and DNA structural elements play an important role in a variety of biological processes, which make them an appealing target for drug discovery. However, traditional protein higher order structural techniques often face challenges when applied to RNA and DNA molecules. Furthermore, these structural techniques are laborious and not amenable to the throughput needed in drug discovery. Moreover, binding techniques capable of higher throughput often do not resolve functional structural elements. In this study, second-harmonic generation (SHG) technology[1] was adapted to study RNA and DNA oligonucleotide conformational changes associated with ligand binding. The technique was applied to three distinct RNA/DNA structural classes, including RNA hairpins, G-quartets, and riboswitches, all of which are known to undergo conformational changes upon binding either protein or small molecule ligands.[2,3] In all three cases, SHG was able to resolve conformational changes in these oligonucleotides sensitively and specifically, in solution and in real time, using nanogram amounts of material. Furthermore, these changes were distinct from conformational changes associated with known nonspecific binders This work demonstrates the broad potential of SHG for studying oligonucleotides and their conformational changes upon interaction with ligands. As SHG offers a powerful, high-throughput screening approach, our results here also open an important new avenue for

identifying novel chemical probes or sequence-targeted drugs that disrupt or modulate DNA or RNA structure and function.

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2 Butko, M. T., Moree, B., Mortensen, R. B. & Salafsky, J. Detection of Ligand-Induced Conformational Changes in Oligonucleotides by Second-Harmonic Generation at a Supported Lipid Bilayer Interface. *Anal Chem* **88**, 10482-10489, doi:10.1021/acs.analchem.6b02498 (2016).

3 Rizvi, N. F. *et al.* Discovery of Selective RNA-Binding Small Molecules by Affinity-Selection Mass Spectrometry. *ACS Chem Biol*, doi:10.1021/acschembio.7b01013 (2018).